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CONIDIAL EVIDENCE FOR HOST RESTRICTION IN *CLAVICEPS PURPUREA*

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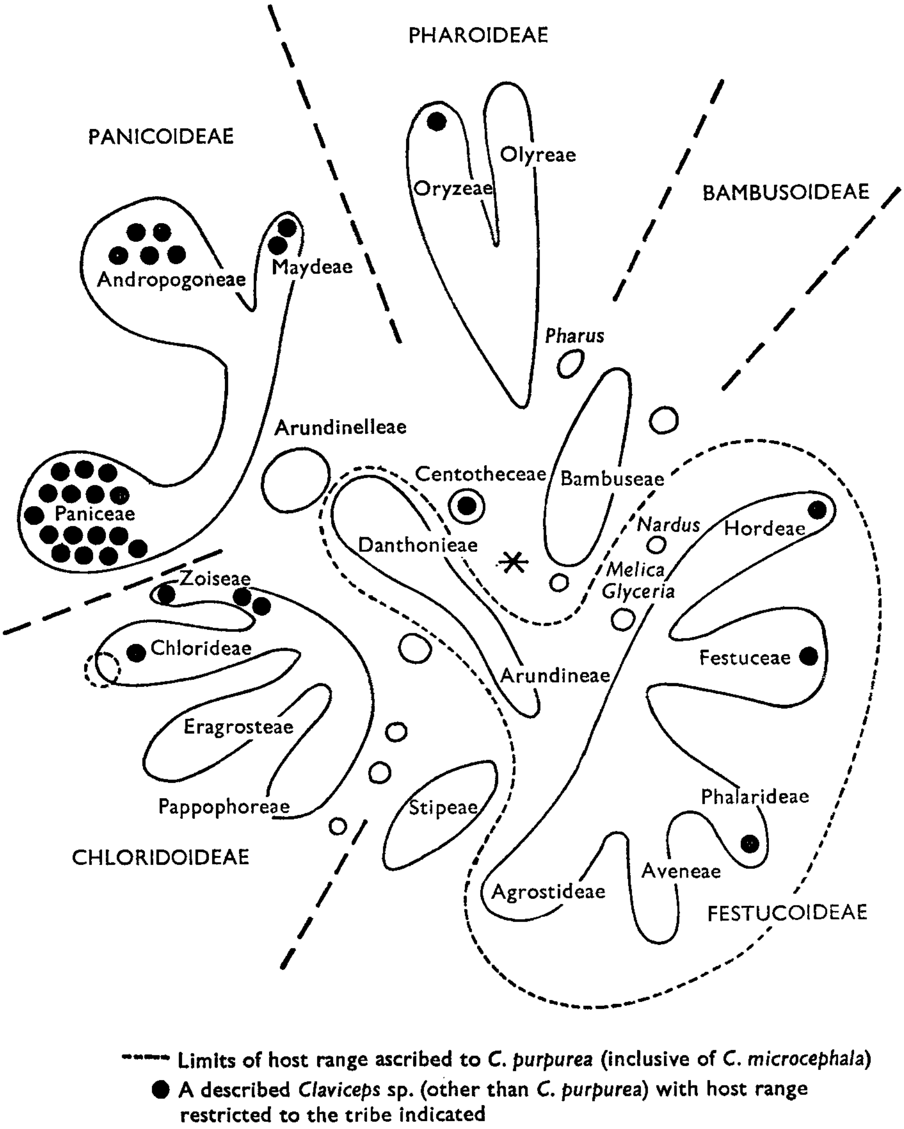
(With Plate 39 and 5 Text-figures)

Graminicolous *Claviceps* spp. other than *C. purpurea* are tribe-specific in their host ranges, whereas *C. purpurea* (including *C. microcephala*) is recorded from grasses belonging to at least seventeen tribes. On the assumption that significant differences in conidial size and shape are indicative of speciation taking place within *C. purpurea*, a comparative study has been made of 101 honeydew samples from thirty-one species of British grasses belonging to twenty-eight genera and thirteen tribes.

Samples showed considerable variation in size and shape of conidia but, after applying Bartlett's test for homogeneity of variance, it is claimed that independent samples collected from different specimens of the same host could reasonably have come from the same population. By contrast, spore populations from different hosts are shown by the analysis of variance and covariance to differ significantly in both size and shape. The results of a transplant experiment, in which conidia were grown in culture from slices of sclerotia with a known size of honeydew conidia, indicate these differences to be primarily under genetic control. Conidial populations from different hosts may therefore be divided into groups by applying Duncan's multiple-range test. The groups are considered sufficiently distinct to provide morphological evidence for host restriction in *C. purpurea*. It is noteworthy that a close similarity exists between the species-composition of these groups and the 'biologic forms' proposed by Stäger from cross-inoculation experiments.

There are thirty-six described species of *Claviceps* (Langdon, 1954; *Bibliography of Systematic Mycology*, 1953 onwards), of which thirty-three occur on Gramineae and three on Cyperaceae. Different species are distinguished by their host ranges and morphological features. *Claviceps* is taxonomically a difficult genus because positive identifications can be made only by considering a number of characters, the determination of which is time-consuming and often accompanied by practical difficulties. The observation of the perfect state can present difficulty because the ergots (sclerotia) must first be germinated, and the requisite germination conditions vary for different species. Unless these conditions are already known for a particular ergot, they have to be determined by trying a number of experimental treatments, a process which may require many sclerotia before success is achieved. In view of the above difficulties the author (Loveless, 1964) explored the possibility of using the honeydew or *Sphacelia* state as an easy means of characterization. Using collections of honeydew from thirty-four Rhodesian grasses, the author recognized thirteen conidial groups on the basis of size and shape of the conidia, considered in conjunction with the host range. Six of these groups corresponded to the six species of *Claviceps* then known to occur in Rhodesia, two have since been described as new species (*C. rhynchelytri* Herd & Loveless, 1965, and *C. fusiformis* Loveless, 1967), and the remaining five would seem to represent *Claviceps* spp. still undescribed but with known hosts.

Of the graminicolous species of *Claviceps* all except *C. purpurea* (Fr.) Tul. are recorded as having a relatively narrow host range restricted to grasses belonging to one particular tribe (Text-fig. 1). Most species have a monogeneric host range, while a few are found on several host genera restricted



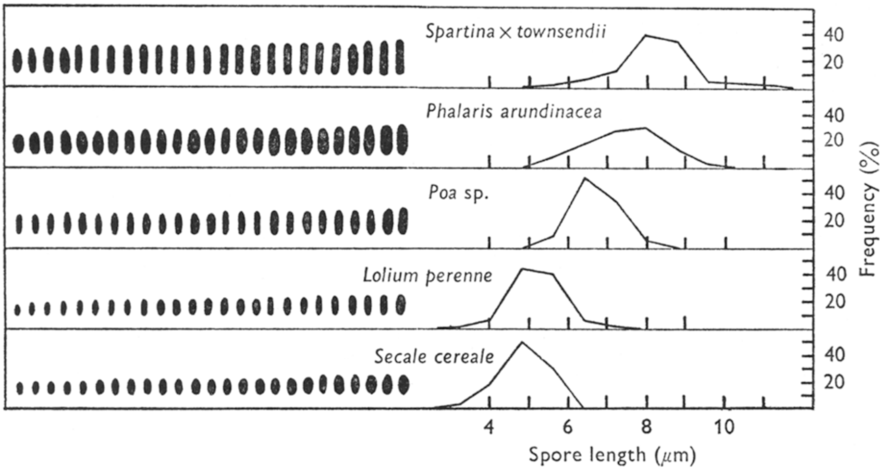
Text-fig. 1. The host relationships of the thirty-three described *Claviceps* spp. occurring on the Gramineae. (Target diagram from Bowden, 1965.)

to a single tribe. Apart from *C. purpurea*, therefore, it would seem that during the evolution of *Claviceps*, development of the ability to parasitize different hosts has been accompanied by morphological changes. The investigation of the honeydews of Rhodesian ergots would endorse this view. *C. purpurea*, however, is apparently unique because it is recorded as having a very wide host range of at least 200 species covering fifty genera and seventeen tribes (Langdon, 1954). Hubbard (1968) classifies British grasses into nineteen tribes, of which thirteen contain species that have been recorded as being ergotized in this country. The causal fungus has always been identified as *C. purpurea* sensu lato (i.e. including *C. microcephala* (Wallr.) Tul. which Petch (1937) reduced to synonymy with *C. purpurea* on the basis of British material). Undoubtedly most of these identifications have been purely arbitrary, and it may therefore be questioned to what extent the exceedingly wide host range of *C. purpurea* is the result of arbitrary identifications rather than of a genuine lack of speciation. It is admitted that the differences between the Hordeeae, Festuceae, Aveneae and Agrostideae (the four tribes to which the majority of British grasses belong) are probably smaller than those between most other grass tribes but, by analogy with the host specialization of other *Claviceps* spp., it is suggested that *C. purpurea* may have been used as a blanket term for several distinct taxa. As a first step towards testing this possibility, a comparative study has been made of the conidial state of *C. purpurea* sensu lato from as many British grasses as could be obtained. The results of this study form the subject of this paper.

MATERIALS AND METHODS

In any comparative work it is necessary to know how much the fungi under consideration vary for the character being studied. When a character differs widely within a population it can only be used for characterization if a sufficient number of samples is used in computing the means to be compared. It became evident that single samples of conidia from different British grasses often showed considerable variation in size and shape. The magnitude of such variation can be gauged from Text-fig. 2, in which sets of twenty-five spores, traced at random from five different grasses, are arranged side by side in order of increasing length. The frequency distribution curves for the spore lengths of the same five samples (also shown in Text-fig. 2) clearly indicate more than one population, but it is impossible to draw any conclusions from spore measurements based only on single samples. The method has therefore been to measure a relatively small number of spores (twenty-five) from a large number of samples, rather than vice versa. Four independent samples for each species of ergotized grass were used, in the hope that this number of replicates would cover the range of possible variation on any one host. Wherever possible the samples were selected to represent widely separated localities and different years of collection. For ten of the grasses included in this investigation it has not been possible to achieve the objective of four independent replicates, and this deficiency limits the use to which the data for these grasses can be put in the subsequent analyses.

The most reliable method of obtaining a sample of conidia is to smear a drop of honeydew directly from the host on to a glass slide. The smear is mounted in cotton blue in lactophenol so that it can be measured when convenient. An alternative method is to soak a dried sclerotium in water, when any conidia adhering to its surface will float off. Although this method works only if the specimen has been collected at the 'right' stage of maturity, its use has enabled herbarium specimens from the Royal Botanic Gardens, Kew, and elsewhere to provide samples more widely distributed in space and time than would otherwise have been possible.



Text-fig. 2. Spore samples from five British grasses to show variation in size and shape of honeydew conidia of *C. purpurea*. The frequency distribution curves refer to the spore lengths of the same five samples.

Using the above two methods for obtaining conidia, 101 samples have been collected from sixty-one localities distributed throughout the British Isles. Collectively these samples represent thirty-one species of grass belonging to twenty-eight genera and thirteen tribes (Table 1). At least two of the species (namely *Puccinellia distans* and *Melica uniflora*) are considered to be new host records for this country, although they have been recorded elsewhere in Europe. It has not been possible to obtain ergotized specimens of all grasses known to be hosts of *C. purpurea* in Britain, but nevertheless the present collections represent a wide coverage of the British grass flora.

From each sample the dimensions of twenty-five random spores were measured on the screen of a Reichert Lanameter at a magnification of × 1250. At this high level of magnification the effect of parallax error is minimal, and mean differences in the order of 1 μm can be readily detected.

Table 1. *Taxonomic distribution of British hosts of* Claviceps purpurea *from which samples of conidia have been collected*

Host	Replicate	Locality	Date	Collector
Chlorideae ('Spartineae')				
Spartina × *townsendii* H. & J. Groves (agg.)	1	Bedhampton, Hants.	10. ix. 66	A. R. Loveless
	2	Newtown, I. of Wight	17. ix. 68	A. R. Loveless
	3	Poole, Dorset	28. ix. 68	A. R. Loveless
	4	Budleigh Salterton, Devon	Jan. 1969	D. J. Marchant
Danthonieae				
Molinia caerulea (L.) Moench	1	Applecross, Ross & Crom.	30. ix. 46	R. W. G. Dennis
	2	Ottery St Mary, Devon	23. viii. 64	A. R. Loveless
	3	Richmond, Surrey	21. ix. 64	A. R. Loveless
	4	Purbrook, Hants.	12. ix. 67	A. R. Loveless
Arundineae				
Phragmites communis Trin.	1	Droitwich, Worcs.	28. x. 27	W. B. Grove & P. G. M. Rhodes
	2	Woolston, Hants.	24. x. 67	A. R. Loveless
	3	Kennyhill, Suffolk	20. x. 68	A. R. Loveless
	4	Portsmouth, Hants.	14. xi. 69	A. R. Loveless
Nardeae				
Nardus stricta L.	1	Balcosta, I. of Unst	25. viii. 52	R. W. G. Dennis
	2	Near Hury, Yorks.	26. viii. 68	F. N. Haynes
	3	Llanddewi Brefi, Cardigan.	31. viii. 69	A. R. Loveless
	4	Two Bridges, Devon	7. ix. 69	L. F. Marchant
Glycerieae				
Glyceria fluitans (L.) R. Br. & hybrid *G.* × *pedicellata* Towns	1	Purbrook, Hants.	17. vii. 66	A. R. Loveless
	2	Salen, I. of Mull	29. vii. 68	R. W. G. Dennis
	3	Barmouth, Merion.	20. vii. 69	P. J. O. Trist
	4	Llanfair Clydogau, Cardigan.	7. ix. 69	A. R. Loveless
Hordeeae				
Agropyron pungens (Pers.) Roem. & Schult.	1	Bedhampton, Hants.	25. ix. 66	A. R. Loveless
	2	Poole, Dorset	28. ix. 68	A. R. Loveless
	3	Holkham, Norfolk	26. ix. 69	D. Yarham
	4	Waldringfield, Suffolk	10. x. 69	G. K. Loveless
Agropyron repens (L.) Beauv.	1	S. Wootton, Norfolk	3. ix. 1876	C. B. Plowright
	2	Waterlooville, Hants.	21. x. 65	A. R. Loveless
	3	Kenilworth, Warwicks.	23. viii. 67	A. R. Loveless
	4	Llanfair Clydogau, Cardigan.	1. ix. 69	A. R. Loveless
Elmyus arenarius L.	1	Near Mold, Flint.	Aug. 1945	C. M. Swaine
	2	Tentsmuir, Fife	23. viii. 68	L. F. Marchant
Hordeum distichon L.	1	Boxworth, Cambs.	10. ix. 68	D. Yarham
H. murinum L.	1	Drayton, Hants.	10. x. 66	A. R. Loveless
	2	Cosham, Hants.	6. viii. 70	A. R. Loveless
Secale cereale L.	1	Scunthorpe, Lincs.	25. viii. 10	C. Crossland
	2	Invergordon, Ross & Crom.	22. viii. 22	A. D. Cotton
	3	Near Westbury, Wilts.	16. viii. 48	S. R. Wiltshire
	4	Iken, Suffolk	7. viii. 69	D. Yarham
Triticum aestivum L.	1	Hertfordshire	Aug. 1964	Unknown
	2	Eyeworth, Beds.	4. viii. 69	G. K. Loveless
	3	Elmswell, Suffolk	Aug. 1969	G. K. Loveless
	4	Near Cambridge, Cambs.	Sept. 1969	M. Noble
Festuceae				
Dactylis glomerata L.	1	Dumfriesshire	Sept. 1894	A. L. Smith
	2	Ham, Surrey	20. ix. 64	A. R. Loveless
	3	Kenilworth, Warwicks.	24. viii. 67	A. R. Loveless
	4	Dale, Pembroke.	7. viii. 68	A. R. Loveless
Festuca arundinacea Schreb.	1	Hackney, London	7. x. 27	R. Melville
	2	Near Surlingham, Norfolk	12. viii. 59	R. W. G. Dennis
	3	Purbrook, Hants.	15. x. 66	A. R. Loveless
	4	Kenilworth, Warwicks.	20. viii. 67	A. R. Loveless
× *Festulolium loliaceum* (Huds.) P. Fourn.	1	Kew, Surrey	10. x. 67	A. R. Loveless
	2	Stour Estuary, Suffolk	24. ix. 68	P. J. O. Trist

Table 1 (*cont.*)

Host	Replicate	Locality	Date	Collector
Lolium perenne L. (including *L. multiflorum* Lam.)	1	Bedhampton, Hants.	2. vii. 66	A. R. Loveless
	2	Kenilworth, Warwicks.	24. viii. 67	A. R. Loveless
	3	Near Milford Haven, Pembroke.	13. viii. 68	A. R. Loveless
	4	Bonnyrigg, Midloth.	Oct. 1969	M. Noble
Poa sp.	1	Bedhampton, Hants.	28. viii. 68	A. R. Loveless
Puccinellia distans (L.) Parl.	1	Bedhampton, Hants.	2. vii. 66	A. R. Loveless
	2	Bedhampton, Hants.	3. vii. 67	A. R. Loveless
	3	Bedhampton, Hants.	27. vii. 69	A. R. Loveless
Brachypodieae				
Brachypodium sylvaticum (Huds.) Beauv.	1	Near Buriton, Hants.	13. x. 68	A. R. Loveless
	2	Near Buriton, Hants.	15. ix. 69	A. R. Loveless
Bromeae				
Bromus racemosus L.	1	Near Immingham, Lincs.	8. x. 66	G. Waterhouse
Meliceae				
Melica uniflora Retz.	1	Near Airlie, Angus	24. viii. 68	L. F. Marchant
Phalarideae				
Anthoxanthum odoratum L.	1	Near Belfast, Co Down	Sept. 1931	F. L. Stephens
	2	Near Airlie, Angus	24. viii. 68	L. F. Marchant
	3	Eling, Hants.	1. x. 68	A. R. Loveless
	4	Llanddewi Brefi, Cardigan.	31. viii. 69	A. R. Loveless
Phalaris arundinacea L.	1	Loch Ness, Inverness.	28. viii. 55	D. A. Reid
	2	Oban, Argyll.	8. ix. 66	R. W. G. Dennis
	3	Funtington, Sussex	28. vii. 68	A. R. Loveless
	4	Llanfair Clydogau, Cardigan.	7. ix. 69	A. R. Loveless
Aveneae				
Arrhenatherum elatius (L). Beauv. ex. J. & C. Presl.	1	Dumfriesshire	Sept. 1894	A. L. Smith
	2	Bedhampton, Hants.	25. vii. 66	A. R. Loveless
	3	Kenilworth, Warwicks.	24. viii. 67	A. R. Loveless
	4	Dale, Pembroke,	5. viii. 68	A. R. Loveless
Deschampsia caespitosa (L.) Beauv.	1	Hillsborough, Co. Down	11. ix. 31	E. M. Wakefield
	2	Purbrook, Hants.	10. viii. 68	A. R. Loveless
	3	Newtown, I. of Wight	28. viii. 69	A. R. Loveless
	4	Llangybi, Cardigan.	1. ix. 69	A. R. Loveless
Holcus lanatus L.	1	Waterlooville, Hants.	23. x. 65	A. R. Loveless
	2	Bedhampton, Hants.	3. vii. 67	A. R. Loveless
	3	Kenilworth, Warwicks.	24. viii. 67	A. R. Loveless
	4	Dale, Pembroke.	5. viii. 68	A. R. Loveless
Agrostideae				
Agrostis canina L.	1	Purbrook, Hants.	10. ix. 67	A. R. Loveless
	2	Bedhampton, Hants.	28. viii. 68	A. R. Loveless
	3	Llanfair Clydagau, Cardigan.	6. ix. 69	A. R. Loveless
	4	Waterlooville, Hants.	10. ix. 69	A. R. Loveless
Alopecurus myosuroides Huds.	1	Without locality	Nov. 1871	J. Buckman
	2	Boxworth, Cambs.	19. ix. 68	D. Yarham
	3	Lidgate, Suffolk	4. xii. 68	G. K. Loveless
A. pratensis L.	1	Near Bromley, Kent	4. ix. 04	B. T. Lowne
	2	Tomich, Inverness.	10. ix. 63	E. R. Wallace
	3	Waterlooville, Hants.	23. x. 65	A. R. Loveless
	4	Bedhampton, Hants.	25. vii. 66	A. R. Loveless
Ammophila arenaria (L.) Link & hybrid × *Ammocalamagrostis baltica* (Fl. ex. Schrad.) P. Fourn.	1	Bournemouth, Hants.	Aug. 1843	C. E. Broome
	2	Benacre Ness, Suffolk	12. ix. 56	C. E. Hubbard
	3	Holkham, Norfolk	1. x. 68	D. Yarham
	4	Holkham, Norfolk	26. ix. 69	D. Yarham
Phleum pratense L.	1	Hillsborough, Co. Down	11. ix. 31	E. M. Wakefield
	2	Purbrook, Hants.	12. ix. 67	A. R. Loveless
	3	Llangybi, Cardigan.	1. ix. 69	A. R. Loveless
	4	Carse of Stirling, Stirl.	Sept. 1969	M. Noble

Table 2. *Spore measurements (in μm) of honeydew conidia of* Claviceps purpurea *from thirty-one British hosts*

(Figures for each replicate based on 25 random spores.)

	Replicate no. 1	2	3	4	Mean dimensions		Length/ breadth ratio
	Length Breadth	Length Breadth	Length Breadth	Length Breadth	Length (mean ± S.E.)	Breadth (mean ± S.E.)	
Hosts with 4 replicates							
Spartina × *townsendii* (agg.)	8·38 × 2·85	8·05 × 2·82	8·34 × 2·83	8·66 × 3·01	8·36 ± 0·12	2·88 ± 0·04	2·9
Molinia caerulea	7·43 × 3·02	7·01 × 2·42	7·20 × 2·54	7·01 × 2·67	7·16 ± 0·10	2·66 ± 0·13	2·7
Phragmites communis	8·53 × 3·15	7·07 × 2·64	7·94 × 3·06	7·18 × 2·96	7·68 ± 0·34	2·95 ± 0·11	2·6
Nardus stricta	8·66 × 3·44	8·26 × 2·59	7·95 × 2·91	8·78 × 3·09	8·41 ± 0·19	3·01 ± 0·18	2·8
Glyceria fluitans and hybrid	6·06 × 3·01	6·88 × 2·69	6·72 × 3·49	6·46 × 3·05	6·53 ± 0·18	3·06 ± 0·16	2·1
Agropyron pungens	5·78 × 2·91	5·81 × 2·69	6·70 × 2·83	6·08 × 2·32	6·09 ± 0·22	2·69 ± 0·13	2·3
A. repens	6·67 × 2·83	6·54 × 2·62	5·38 × 2·34	5·50 × 2·24	6·02 ± 0·34	2·51 ± 0·14	2·4
Secale cereale	5·78 × 2·91	5·81 × 2·58	5·87 × 3·10	5·58 × 2·93	5·76 ± 0·06	2·88 ± 0·11	2·0
Triticum aestivum	5·94 × 2·61	5·74 × 2·52	5·66 × 2·59	5·92 × 2·66	5·82 ± 0·07	2·59 ± 0·03	2·2
Dactylis glomerata	6·90 × 2·91	5·89 × 2·26	6·11 × 2·61	6·48 × 2·37	6·35 ± 0·22	2·54 ± 0·15	2·5
Festuca arundinacea	6·48 × 3·30	6·08 × 2·77	5·94 × 2·80	6·03 × 2·88	6·13 ± 0·12	2·94 ± 0·12	2·1
Lolium perenne	5·46 × 2·69	4·90 × 2·27	5·17 × 2·38	6·32 × 2·74	5·46 ± 0·31	2·52 ± 0·11	2·2
Anthoxanthum odoratum	6·08 × 2·32	5·89 × 2·24	5·02 × 2·26	6·53 × 2·21	5·88 ± 0·32	2·26 ± 0·02	2·6
Phalaris arundinacea	7·49 × 3·42	9·07 × 3·38	7·63 × 3·47	7·29 × 3·65	7·87 ± 0·41	3·48 ± 0·06	2·3
Arrhenatherum elatius	6·00 × 2·70	5·68 × 2·70	5·07 × 2·56	5·89 × 3·23	5·66 ± 0·21	2·80 ± 0·15	2·0
Deschampsia caespitosa	7·71 × 3·36	7·95 × 3·50	7·77 × 4·15	6·80 × 3·13	7·56 ± 0·26	3·54 ± 0·22	2·1
Holcus lanatus	6·45 × 2·61	6·91 × 2·51	5·94 × 2·74	6·37 × 2·45	6·42 ± 0·20	2·58 ± 0·06	2·5
Agrostis canina	7·39 × 3·54	8·10 × 3·66	8·18 × 3·46	8·83 × 3·33	8·12 ± 0·29	3·50 ± 0·07	2·3
Alopecurus pratensis	6·61 × 3·18	7·89 × 3·10	6·50 × 2·40	7·70 × 2·74	7·17 ± 0·36	2·86 ± 0·18	2·5
Ammophila arenaria and hybrid	5·62 × 2·53	6·35 × 2·96	5·71 × 2·80	5·77 × 2·88	5·86 ± 0·17	2·79 ± 0·09	2·1
Phleum pratense	8·42 × 3·55	7·81 × 2·82	8·35 × 3·41	7·18 × 3·39	7·94 ± 0·29	3·29 ± 0·16	2·4
Hosts with 3 replicates							
Puccinellia distans	6·86 × 2·85	6·83 × 2·77	6·54 × 3·11	—	6·75 ± 0·13	2·91 ± 0·10	2·3
Alopecurus myosuroides	6·46 × 2·66	5·90 × 2·42	5·70 × 2·50	—	6·02 ± 0·23	2·52 ± 0·07	2·4
Hosts with 2 replicates							
Hordeum murinum	7·60 × 2·34	7·66 × 2·46	—	—	7·63 ± 0·03	2·40 ± 0·06	3·2
Elymus arenarius	5·46 × 3·02	6·30 × 2·86	—	—	5·88 ± 0·42	2·94 ± 0·08	2·0
× *Festulolium loliaceum*	6·08 × 2·77	5·66 × 2·83	—	—	5·87 ± 0·21	2·80 ± 0·03	2·1
Brachypodium sylvaticum	8·00 × 2·29	6·74 × 2·10	—	—	7·37 ± 0·63	2·19 ± 0·09	3·4
Hosts with 1 replicate							
Hordeum distichon	6·67 × 2·29	—	—	—	6·67 ± 0·26	2·29 ± 0·07	2·9
Poa sp.	6·90 × 2·62	—	—	—	6·90 ± 0·09	2·62 ± 0·04	2·6
Bromus racemosus	7·27 × 3·47	—	—	—	7·27 ± 0·17	3·47 ± 0·08	2·1
Melica uniflora	5·50 × 1·81		—	—	5·50 ± 0·17	1·81 ± 0·07	3·0

RESULTS

Table 2 summarizes the spore measurements of the 101 samples listed in Table 1. It is desired to use the data of Table 2, supplemented where necessary by further data, to answer the following four questions. (1) Can spore samples from the same host be regarded as belonging to a single population? (2) Do spore samples from different hosts differ significantly in length or breadth or both? (3) If there are significant differences in spore size between hosts, can such differences be attributed to the genotype of the fungus rather than to the environment of the host? (4) Can conidial populations from different hosts be divided meaningfully into a series of groups based on spore size and shape? These four questions form the sequence for describing the following results.

Variation between different spore samples from the same species of host

If spore size is to be used for comparing populations of spores from different hosts, it is necessary at the outset to establish that the variation in spore measurements from individual hosts is consistent with a homogeneous population. The null hypothesis is therefore proposed that no significant difference exists in the variability of spore size between independent samples collected from different specimens of the same host. For this purpose spore length alone is considered because, although both length and breadth were measured, the differences between spores are greatest in this dimension. A suitable and appropriate test of significance for this type of problem is Bartlett's test for homogeneity of variance because it is sensitive not only to inequality of variance but also to departure from normality of distribution. Application of Bartlett's test, therefore, will not only test the above hypothesis but also check that the frequency distributions for the individual hosts are normal. This assumption should be checked because, in the following section, the data which are being tested here for constancy of variance will be examined by the technique of the analysis of variance. Although the analysis of variance is a 'robust' procedure, it is nevertheless essential to check the assumption of approximate normality for the individual levels if the analysis is to give valid conclusions.

Since Bartlett's test is only appropriate when more than two variances are to be compared, the twenty-three hosts in Table 1 which satisfy this requirement will be used. The calculated values of Bartlett's chi-squared for these hosts are given in Table 3, from which it will be seen that for nineteen of the twenty-three hosts the values are not significant. For four hosts, however, the values are significant at levels varying from 5 % to just below 0·1 %, which means that in these four hosts there is evidence of heterogeneity of variance. Despite these four significant values, it is nevertheless claimed that the results as a whole indicate that the population variances are homogeneous on individual hosts. Since twenty-three hosts are included in Table 3, at least one significant departure from the null hypothesis can be expected purely by chance. Furthermore, it should be pointed out that collections of any fungus from widely separated localities may be expected to vary more than samples from a restricted locality, and in this investigation a deliberate effort has been made to collect samples from localities as widely separated as possible. With the present data, therefore, Bartlett's test is more likely to reject the null hypothesis when it is actually true than vice versa. In the circumstances it could be argued that the highly significant chi-squared values for *Lolium perenne* and *Holcus lanatus* give spurious results, because in each case the high chi-squared value is caused by the inclusion of one sample which diverges widely in its variance from the other three samples in the set. Therefore, on the evidence obtained here, homogeneity of variance on individual hosts will be accepted as a working hypothesis.

Table 3. *Use of Bartlett's test for comparing the variances of spore-length measurements of independent samples from the same host*

	Value of Bartlett's chi-squared		Value of Bartlett's chi-squared
Spartina × *townsendii*	6·73	*Puccinellia distans*	1·29
Molinia caerulea	8·09*	*Anthoxanthum odoratum*	0·55
Phragmites communis	0·80	*Phalaris arundinacea*	8·95*
Nardus stricta	2·93	*Arrhenatherum elatius*	2·07
Glyceria fluitans and hybrid	5·05	*Deschampsia caespitosa*	5·24
Agropyron pungens	5·27	*Holcus lanatus*	11·81**
A. repens	3·37	*Agrostis canina*	6·98
Secale cereale	2·20	*Alopecurus myosuroides*	1·54
Triticum aestivum	5·30	*Alopecurus pratensis*	6·66
Dactylis glomerata	6·95	*Ammophila arenaria* and hybrid	5·21
Festuca arundinacea	3·15	*Phleum pratense*	2·04
Lolium perenne	16·82***		

* Significant at the 5 % level. ** Significant at the 1 % level. *** Significant at the 0·1 % level.

Table 4. *Analysis of variance of spore length and of spore breadth with respect to host*

Source of variation	Degrees of freedom	Sum of squares		Mean square		*F*	
		Length	Breadth	Length	Breadth	Length	Breadth
Between hosts	26	85·7235	11·4034	3·2970	0·4385	13·81***	7·48***
Error	71	16·9579	4·1675	0·2388	0·0586	—	—
Total	97	102·6814	15·5709	—	—	—	—

*** Significant at the 0·1 % level.

Variation in size and shape of spores from different species of host

If it is assumed that spore samples from individual host species could reasonably have been obtained from the same population, the next step is to decide whether the variation in spore size shown by different species of host is significantly greater than that shown within individual species. Variation in spore length and spore breadth with respect to host is analysed in Table 4. The analysis of variance excludes the four hosts for which one estimate only of the population mean and variance is available, thus giving 26 degrees of freedom for the variation due to differences between hosts. The calculated values of F for the between-hosts/error comparisons are both highly significant ($P < 0{\cdot}001$), in spite of the fact that the comparisons include the data for the four hosts (*Molinia caerulea*, *Lolium perenne*, *Phalaris arundinacea* and *Holcus lanatus*) which show variation between samples additional to that within samples. The inclusion of these four hosts increases disproportionately the error mean square of the analyses.

In addition to differing in size, however, there is a strong impression from even cursory observation that conidial populations may differ also in shape. For example, whereas the spores of *Lolium perenne* (Pl. 39, fig. 3) are the same shape as, but smaller than, those of *Phalaris arundinacea* (Pl. 39, fig. 4), the spores of *Brachypodium sylvaticum* (Pl. 39, fig. 5) are approximately the same length as, but narrower than, those of *Phalaris arundinacea*. The mean length/breadth ratios for *L. perenne*, *P. arundinacea* and *B. sylvaticum* are 2·2, 2·3 and 3·4 respectively. Using the same data as for Table 4, it is possible to test for differences in spore shape by performing a covariance F-test (Snedecor & Cochran, 1968, p. 424) for spore length adjusted to breadth (Table 5). The calculated value of F, though less than that for spore length alone, is still highly significant ($P < 0{\cdot}001$).

Table 5. *Covariance F-test for comparing spore shape (i.e. length adjusted for breadth) with respect to host*

(x = length, y = breadth.)

Source of variation	Degrees of freedom	Sums of squares and products x^2	xy	y^2	Adjusted means Degrees of freedom	Sum of square	Mean square	F
Hosts	26	85·7235	17·8940	11·4034	—	—	—	—
Error	71	16·9579	2·9580	4·1675	70	14·8584	0·2123	—
Total	97	102·6814	20·8520	15·5709	96	74·5770	—	—
Hosts†	—	—	—	—	26	59·8986	2·3038	10·85***

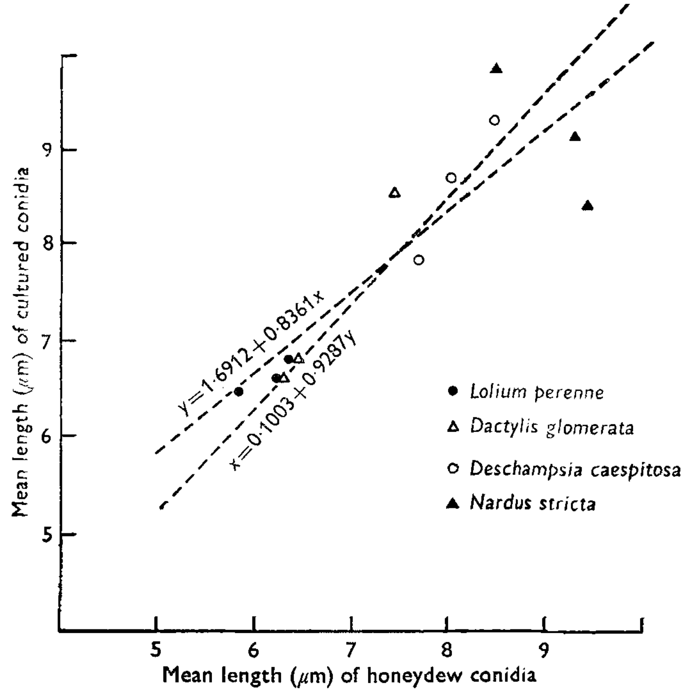
*** Significant at the 0·1 % level.
† Adjusted for the average within-hosts regression.

Since the results shown in Tables 4 and 5 are based on samples from widely separated localities, it may be concluded that spore populations from different hosts differ widely in both size and shape.

Evidence that differences in spore size are due largely to genetic variation

In view of the fact that the habitat of a parasitic fungus is its host, it could reasonably be argued that any variation in size and shape between conidia from different hosts might be explained by the differential response of the fungus to different habitats. In the case of *C. purpurea* the formation of conidia on a host plant is always accompanied by the production of honeydew, and the osmotic potential of this secretion naturally suggests itself as an environmental factor that might vary widely on different hosts and so influence spore size and shape. Before the highly significant results of the previous section can be taken to imply genetic diversity in *C.*

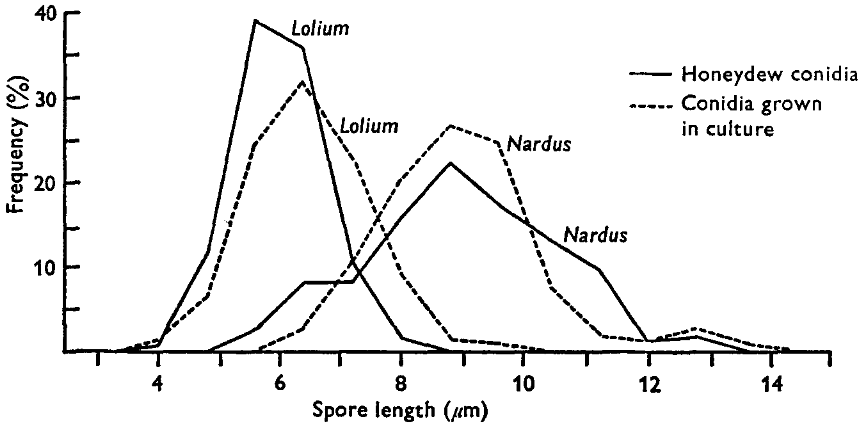
purpurea, it is necessary to show that the observed morphological differences are primarily under genetic control. The method adopted was to perform a 'transplant experiment' in which the effects of the environment are standardized. This approach is possible because *C. purpurea*, although ecologically an obligate parasite, can be readily grown on a wide range of culture media. The mycelia usually produce abundant conidia but these are not formed in a sugary exudate comparable to honeydew. Sclerotia also fail to form in culture.



Text-fig. 3. The relationship between the mean length of honeydew conidia and of conidia grown in culture from the same sclerotia.

The transplant experiment consisted of growing cultures from sclerotia with a known size of honeydew conidia, and then comparing the sizes of the conidia produced in culture with those of the original honeydews. Spore length was used as the criterion of size because, as previously mentioned, differences between spores are greatest in this dimension. Sclerotia of *Lolium perenne*, *Dactylis glomerata*, *Deschampsia caespitosa* and *Nardus stricta* were chosen, because honeydews from these hosts cover the observed range in conidial size. For each host three sclerotia, collected from different localities, were used. From each sclerotium a honeydew mount was prepared by soaking it in water on a slide, and a mycelial culture was then established by plating a sterile slice of the sclerotium on potato dextrose agar. The cultures were maintained at 25 °C and after 21 days, when sporulation was profuse, conidial mounts were made. Length measurements were made of fifty random spores from each of the twelve honeydew mounts and of the corresponding twelve mounts of conidia produced in culture. The reason for measuring fifty (instead of the standard twenty-five) spores to estimate the mean size was to cover the possibility that spores produced in culture might be more variable than those produced in honeydew.

If the observed variation shown by honeydew conidia is largely environmental, there should be little or no correlation between the mean spore lengths of the honeydew conidia and those of the conidia produced in a constant cultural environment. If the variation is largely genetic, however, there should be a high degree of correlation. The scatter diagram (Text-fig. 3) relating the mean spore lengths for the above twelve pairs of mounts shows a highly significant correlation ($r = +0{\cdot}88$, $P < 0{\cdot}001$). The slopes of the two regression lines (40° for y on x, and 43° for x on y) approximate to 45°, which, since both the x and y axes are drawn to the same scale, would be the slope obtained if an exactly proportionate relation had existed between the spore lengths before and after culturing. The conclusion that spore size is primarily under genetic control is also borne out by comparing the standard deviations of the spore-length measurements. The frequency distribution curves (Text-fig. 4) for *Lolium perenne* and *Nardus stricta* (which have the shortest and longest conidia of the four hosts considered) show that the standard deviations, though clearly different for the two hosts, are similar for each host, irrespective of whether the conidia are obtained from sclerotia or cultures.

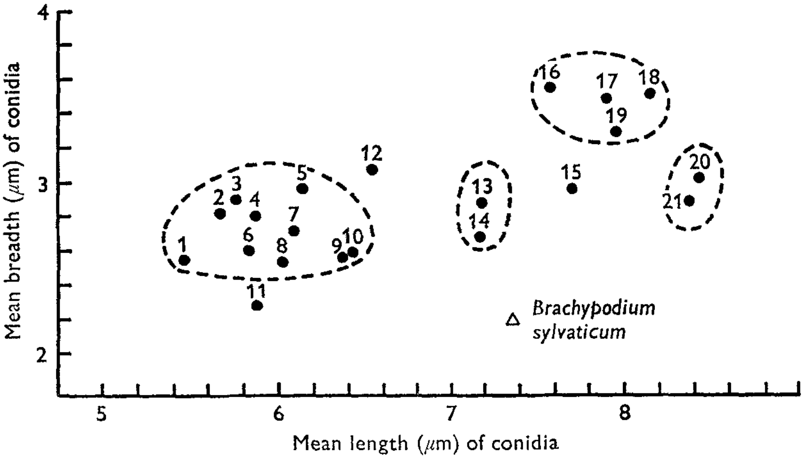


Text-fig. 4. Frequency distribution curves for length measurements of conidia from *Lolium perenne* and *Nardus stricta* before and after culturing. Each curve is based on three replicates.

The results of the transplant experiment therefore justify the use of honeydew conidia to indicate genetic diversity in *C. purpurea*.

Possibility of arranging conidial populations from different hosts into groups

Having established (1) that significant differences in size exist between honeydew conidia collected from different grasses and (2) that these differences are primarily genetic, it remains to compare the mean conidial sizes of the honeydews investigated. Because the data were obtained to see whether conidial size provided any evidence for host restriction within *C. purpurea*, no specific comparisons can be singled out as being more important than others. On the assumption that significant differences in conidial size can be used to indicate speciation within *C. purpurea*, a test is required for dividing the observed means into groups that are similar in size and shape. Such groups can be presumed to represent 'segregates' of *C. purpurea*, each with a host range circumscribed by the means within the group. The logic of this reasoning is consistent with the expectation of groups, and therefore a multiple-range test can justifiably be used for finding them. Because it was not possible to achieve the objective of four independent replicates for all the ergotized hosts, it was decided to include in the multiple range test only the twenty-one hosts with a complete set of data (i.e. those for which four separate estimates of the population mean and variance are available). In view of the large number of comparisons between pairs of means for twenty-one hosts, Duncan's multiple-range test (Duncan, 1955) was chosen in preference to Newman–Keuls' test because it allows the experimenter to commit fewer type II errors, i.e. those which result in the acceptance of a null hypothesis when it is actually false.



Text-fig. 5. The mean spore sizes of honeydew conidia from different hosts grouped according to Duncan's multiple-range test applied at $P = 0\cdot05\,\%$. The mean size of conidia of *Brachypodium sylvaticum* (based on only two estimates and therefore excluded from the test) is shown for comparison. Key to numbers: 1, *Lolium perenne*; 2, *Arrhenatherum elatius*; 3, *Secale cereale*; 4, *Ammophila arenaria*; 5, *Festuca arundinacea*; 6, *Triticum aestivum*; 7, *Agropyron pungens*; 8, *Agropyron repens*; 9, *Dactylis glomerata*; 10, *Holcus lanatus*; 11, *Anthoxanthum odoratum*; 12, *Glyceria fluitans*; 13, *Alopecurus pratensis*; 14, *Molinia caerulea*; 15, *Phragmites communis*; 16, *Deschampsia caespitosa*; 17, *Phalaris arundinacea*; 18, *Agrostis canina*; 19, *Phleum pratense*; 20, *Nardus stricta*; 21, *Spartina* × *townsendii*.

Because populations of honeydew conidia differ in shape as well as in size, the present analysis involves grouping the hosts according to both length and breadth. After applying Duncan's test, at a probability level of 0·05 %, the most rational method of grouping the hosts is pictured graphically in Text-fig. 5. Hosts enclosed by a dotted line are asserted to have conidia which are not heterogeneous in size or shape. It will be noted that three hosts are not included in any group. *Phragmites communis* (dot 15), in particular, was difficult to place because it showed a marginal relationship with each of the three surrounding groups; it probably represents a group in its own right. *Anthoxanthum odoratum* (dot 11) and *Glyceria fluitans* (dot 12) lie just outside the large group enclosing dots 1–10. *A. odoratum* is separated on account of the narrowness of its conidia and may represent a separate group. There is a stronger case for regarding *G. fluitans* as distinct because its conidia are both longer and wider than any member of this group.

Although the results of the above analysis justify the division of *C. purpurea* into conidial groups, there is no suggestion that the groups can be regarded as taxonomic categories. The delimitation of taxonomic categories should be based on the correlation of as many characters as possible. When the conidial characters considered here can be correlated with other characters, it is likely that there will be more groups than those defined here. The author has unpublished evidence that the ergots of *Spartina anglica* and *Nardus stricta*, which are here included together in the same conidial group (dots 20–21), differ in other respects and almost certainly represent different taxonomic categories.

CONCLUSIONS

This investigation was undertaken to see whether the conidial state of ergotized grasses provided any evidence for host restriction within *C. purpurea* sensu lato. If the variation in size of conidial samples collected from different grass hosts were not statistically significant, then the conidial state would provide no basis for doubting the very wide host range ascribed to this fungus. However, since highly significant differences in conidial size have been shown to exist, the taxonomic status of *C. purpurea* remains doubtful and the extent of its reported host range can still be questioned. Although this investigation has achieved its limited objective of demonstrating the existence of conidial groups which are host specific, it is nevertheless appropriate to interpret these groups in the light of what is known about the host specialization of *C. purpurea*.

There are conflicting reports in the literature concerning the ability of this fungus to pass from one host species to another. As a result of inoculating a large number of grass hosts with isolates of *C. purpurea* from several different hosts, Stäger concluded that this fungus is divided into several 'biologic forms' (or formae speciales as they would now be called), each with its own distinct host range. From his earlier work Stäger, (1903, 1905) recognized four special forms, *secalis*, *milii*, *lolii* and *glyceriae* (with the possibility of a fifth from *Anthoxanthum odoratum*) for *C. purpurea* sensu Tulasne, i.e. exclusive of *C. microcephala* (Wallr.) Tul. He later queried the validity of his f.sp. *lolii* (Stäger, 1922) and added the ergot of *Phalaris arundinacea* as a new form named *natans Phalaris arundinaceae* (Stäger, 1923). Two special forms of *C. microcephala* were recognized by Stäger: the typical form from *Phragmites communis* and other hosts (Stäger, 1903), and a second form with *Poa annua* as its only host (Stäger, 1908). However, Campbell (1957) found no evidence for the special forms proposed by Stäger. He grew 421 isolates of *C. purpurea* from thirty-eight different host species in agar culture to obtain samples of conidia. These were used to inoculate rye (Prolific), wheat (Thatcher) and barley (Newal) plants growing in the greenhouse. With one exception, all cultures infected the three host species tested. Campbell also used honeydew from rye to inoculate forty-six grass species growing in the field as well as in the greenhouse. Every one of the grasses became infected in both locations. On the basis of his results, Campbell denies the existence of special forms of *C. purpurea* and suggests that Stäger's techniques of inoculation were at fault.

In the context of the contradictory reports of Stäger and Campbell it is interesting to note that the present results provide some measure of support for Stäger's findings. Thus, there is a close parallel between Stäger's special forms *secalis*, *milii*, *glyceriae* and *natans Phalaris arundinaceae* and the conidial groups represented in Text-fig. 5 by dots 1–10, by the triangle indicating the position of *Brachypodium sylvaticum* (which Stäger claims to be a host of f.sp. *milii*), by the isolated dot 12, and by dots 16–19. Stäger's uncertainty as to whether to recognize the ergot on *Anthoxanthum odoratum* as a separate special form, and whether to merge his f.sp. *lolii* into the much larger f.sp. *secalis*, is reflected in similar queries about the delimitation of conidial groups. Here it is tentatively concluded that there is no case for separating the conidia of *Lolium* ergot as a distinct group, but that there is justification for regarding the conidia of *Anthoxanthum* ergot as a separate group. The explanation for the conflicting results of Stäger and Campbell is unknown, but the paradox draws attention to the importance of knowing how infection takes place in nature.

The host range of *C. purpurea* in nature is directly related to the agricultural problem of whether wild grasses provide a source of inoculum for cereals and forage grasses. With the recent increase in the occurrence of ergot on wheat in this country, particularly in East Anglia, a knowledge of the biologic status of *C. purpurea* is likely to become a matter of vital importance. Because physiologic races almost certainly exist in such a polymorphic fungus as *C. purpurea*, detailed morphological studies should logically precede cross-inoculation experiments. By analogy with *Puccinia graminis* Pers. it is claimed that, before there can be a rational basis for separating *C. purpurea* into categories based on pathogenicity of different host plants, any morphologically distinguishable categories that may exist should first be recognized. This paper deals exclusively with the conidial state but, with the assistance of Miss Janet Peach, work on the perfect state is also in progress. When the data for both the imperfect and perfect states are combined, it is probable that a number of morphological categories will be distinguishable. These should provide the background against which to interpret the results of cross-inoculation experiments which reproduce as nearly as possible the mode of natural infection.

For helpful discussion during this investigation I am grateful to my colleagues Mr J. F. Ainsworth, Mr H. G. Chappell, Dr R. D. Eaton and, especially, Dr J. W. Dancer, who has also advised me on the statistical presentation of the results. My thanks are due to Mr F. N. Haynes, Mr G. K. Loveless, Mr D. J. Marchant, Mr L. F. Marchant, Dr Mary Noble, Mr P. J. O. Trist and Mr D. Yarham for collecting ergots in localities remote from Portsmouth, while Dr R. W. G. Dennis kindly allowed me to consult the herbarium specimens of *C. purpurea* (many of which were collected by him) which are in his charge at Kew. Finally I must thank Miss Janet Peach for technical assistance, and my wife for her continued help throughout the investigation.

REFERENCES

BARTLETT, M. S. (1937). Some examples of statistical methods of research in agriculture and applied biology. *Journal of the Royal Statistical Society, Supplement.* **4**, 137–183.

BOWDEN, B. N. (1965). Modern grass taxonomy. *Outlook on Agriculture* **4**, 243–253.

CAMPBELL, W. P. (1957). Studies on ergot infection in gramineous hosts. *Canadian Journal of Botany* **35**, 315–320.

DUNCAN, D. B. (1955). Multiple range and multiple *F* tests. *Biometrics* **11**, 1–42.

HERD, G. W. & LOVELESS, A. R. (1965). Studies on Rhodesian ergots. III. *Claviceps rhynchelytri* sp.nov. and *Claviceps digitariae* Hansford. *Kirkia* **5**, 21–24.

HUBBARD, C. E. (1968). *Grasses*, 2nd ed. Harmondsworth: Penguin Books Ltd.

LANGDON, R. F. N. (1954). The origin and differentiation of *Claviceps* species. *University of Queensland Papers, Department of Botany* **3**, 61–68.

LOVELESS, A. R. (1964). Use of the honeydew state in the identification of ergot species. These *Transactions* **47**, 205–213.

LOVELESS, A. R. (1967). *Claviceps fusiformis* sp.nov., the causal agent of an agalactia of sows. These *Transactions* **50**, 15–18.

PETCH, T. (1937). More about *Claviceps*. *The Naturalist, Hull*, no. 961, pp. 25–28.

SNEDECOR, G. W. & COCHRAN, W. G. (1968). *Statistical methods*, 6th ed. Ames: The Iowa State University Press.

STÄGER, R. (1903). Infectionsversuche mit Gramineen-bewohnenden *Claviceps*-Arten. *Botanische Zeitung* **61**, 111–158.

STÄGER, R. (1905). Weitere Beiträge zur Biologie des Mutterkorns. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* (*Abteilting II*) **14**, 25–32.

STÄGER, R. (1908). Zur Biologie des Mutterkorns. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* (*Abteilting II*) **20**, 272–279.

STÄGER, R. (1922). Beiträge zur Verbreitungsbiologie der *Claviceps*-Sklerotien. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* (*Abteilting II*) **56**, 329–339.

STÄGER, R. (1923). Impfversuche mit dem Mutterkorn des Weizens. *Mitteilungen der Naturforschenden Gesellschaft, Bern, 1922*, pp. 11–20.

EXPLANATION OF PLATE 39

Figs. 1, 2. A comparison between conidia from *Secale cereale* (Fig. 1), the type host of *C. purpurea*, and those from *Spartina anglica* (Fig. 2).

Figs. 3–5. The variation in shape of conidia from different hosts. Fig. 3 *Lolium perenne* (length/breadth ratio 2·2). Fig. 4, *Phalaris arundinacea* (length/breadth ratio 2·3). Fig. 5, *Brachypodium sylvaticum* (length/breadth ratio 3·4).

Figs. 6, 7. The variation that may exist between spores in a single smear of honeydew. Fig. 6, *Dactylis glomerata*. Fig. 7, *Nardus stricta*.

All figures × 1040.

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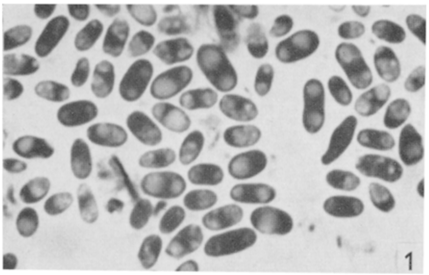
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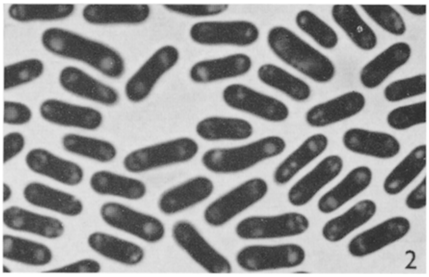
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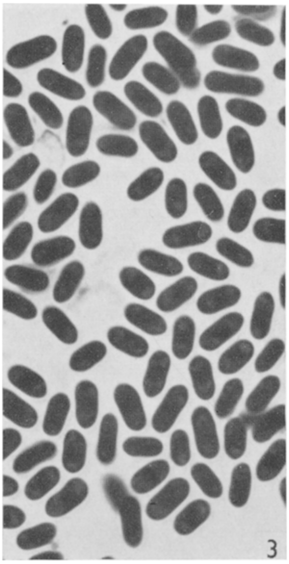
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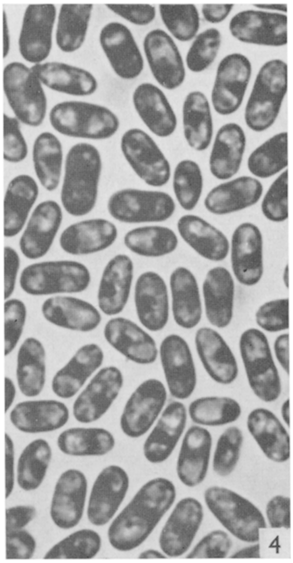
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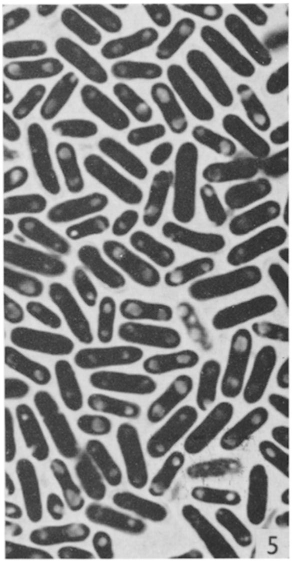
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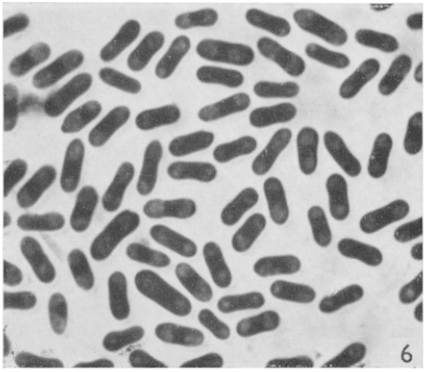
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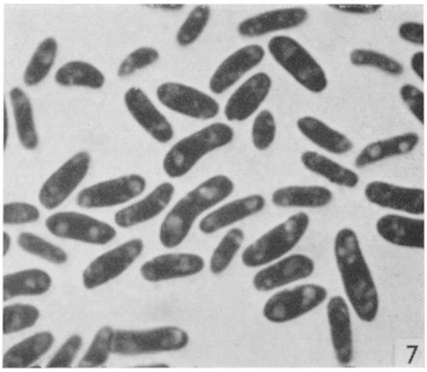
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